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Original Article

DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-UHPLC METHOD FOR THE ESTIMATION OF IMEGLIMIN HYDROCHLORIDE USED FOR THE TREATMENT OF METABOLIC DISORDER DIABETES MELLITUS

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ABSTRACT

Objective: The present work was aimed to develop and validate a novel, simple, rapid, consistent, and sensitive stability indicating reverse phase ultra-high-performance liquid chromatographic (RP-UHPLC) method for the determination of oral anti-diabetic drug Imeglimin Hydrochloride in bulk and pharmaceuticals dosage form as per the technical recommendations of International Council for Harmonization guidelines for human use pharmaceutical (ICH-Q2(R1)).

Methods: A novel simple, selective, stable and sensitive stability indicating method which used isocratic RP-UHPLC methodology for the quantitative measurement of Imeglimin Hydrochloride was developed. The chromatographic separation of Imeglimin Hydrochloride was achieved on RP-UHPLC equipped with Hypersil gold ODS endcapped column of dimensions (150x4.6 mm, 3micron) using isocratic elution with a mobile phase consisting of water: acetonitrile in a ratio of (15:85% v/v) at a flow rate of 1 ml/min with an injection volume of 20 µl. All measurements are done on 240 nm using eight channels Dionex Ultimate 3000 PDA detector equipped with chromeleon data acquisition system for data integration. Validation of the proposed method was carried out according to the (ICH-Q2 (R1)) guidelines.

Results: The retention time of the Imeglimin Hydrochloride was found to be 3.831 with excellent absorbance sensitivity at 240 nm wavelength. The linear regression equation was found to be y = 3199x+1605.5 with a correlation coefficient (R^2)>0.999 which shows excellent linear correlation. Specificity, linearity, precision, accuracy robustness, LOD and LOQ were determined for method validation and results were found to be well within recommended limits as per ICH guidelines.

Conclusion: The proposed stability indicating reverse phase ultra-high-performance liquid chromatographic (RP-UHPLC) method was found to be fast, affordable, robust, precise and specific for estimation of Imeglimin Hydrochloride in pharmaceutical dosage form.

Keywords: Imeglimin hydrochloride, RP-UHPLC, Development, Validation, Stability indicating

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INTRODUCTION

Type 2 diabetes is characterized as a metabolic disorder recognized as a serious public health problem with a considerable impact on human life and health expenditure. Rapid economic development and urbanization have led to rising burden of diabetes in many parts of the world [1]. The prevalence of type 2 diabetes mellitus is increased tremendously worldwide in last several decades along with its rising risk factors of impaired metabolism, obesity and sedentary lifestyles [2, 3]. The prevalence of T2DM which currently affects more than 380 million people worldwide is expected to increase to more than 592 million people by the year 2035 [4, 5]. Various organizations both public and private have committed lot of time, energy and resources for the treatment, prevention and education in order to solve this global problem.

Several oral anti-diabetics were developed to combat the pathophysiology of T2DM but with some limitations in usage due to various adverse effects such as hypoglycemia (Sulfonylureas eg. Glibenclamide, Metiglinides, Thiozolidinediones GLP-1 agonists and Insulin), gastrointestinal (Metformin and voglibose), lactoacidosis (Metformin), weight gain (Sulfonylureas eg. Glipizide, Metiglinideseg. Repaglinide, Thiozolidinediones GLP-1 agonists and Insulin), edema (Thiozolidinedioneseg. Pioglitazone), risk of pancreatitis (GLP-1 receptor agonists), urinary tract infections (SGLT2 inhibitors eg. Dapagliflozin) [6]. Even after the availability of several anti-diabetic agents none of them targets all three components of diabetes pathophysiology such as excessive glucose production due gluconeogenesis, increased insulin resistance and lack of insulin production due to pancreatic β-cell dysfunction [6-8].

Imeglimin Hydrochloride (also called Imeglimin) a recently approved oral anti-diabetic drug is a first of the class of tetrahydratriazone drug called "glimins" [9]. Chemically Imeglimin Hydrochloride is-(R)-6-imino-N,N,4-trimethyl-1,4,5,6-tetrahydro-1,3,5-triazin-2-amine hydrochloride. Molecular formula is $C_6H_{14}ClN_5$ with molecular weight of 191.66 g/mol. It shows basic pKa value around 10.21. Several pivotal phase III trials have been completed with evidence of statistically significant glucose lowering and favorable safety and tolerability profile including the lack of severe hypoglycemia. Imeglimin has primary site of action at the aerobic cells of mitochondria and known to improve bioenergetics of mitochondria, prevents apoptosis of pancreatic β-cell and protects β-cell function [10-13]. Imeglimin also decreases excessive hepatic glucose production as a result of preventing gluconeogenesis, increases glucose stimulated insulin secretion by pancreatic β-cell and increases glucose uptake by muscles [8, 14, 15]. Despite of structurally similarity to bigunide drug eg. Metformin but it does not cause lacto acidosis mainly due to its unclear and distinct mechanism of action to inhibit basal mitochondrial respiration and ATP production unlike inhibition of complex I and V by metformin [16, 17]. Overall Imeglimin appears to target a key root cause of T2DM defective cellular energy metabolism.

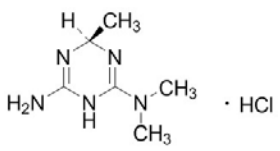

Fig. 1: Structure of imeglimin hydrochloride

In detailed literature survey it was found that there was no stability indicating method for Imeglimin reported till date in the literature but only one method is reported for estimation of Imeglimin by

HPLC. The proposed investigation aims at developing and validating rapid, simple sensitive, accurate and reproducible stability indicating RP-UHPLC method that can be utilized for routine analysis of Imeglimin in bulk and pharmaceuticals. To develop proposed method very advanced and sophisticated RP-UHPLC equipped with endcapped ODS gold column, quaternary pump, very sensitive PDA detector and chromeleon chromatography management system software for data acquisition by thermo fischer scientific was used. This RP-UHPLC produces excellent symmetric sharp peak with very minimal tailing, sensitivity, resolution, high theoretical plate count, low retention time and can withstand high pressure of 620 bar with less noisy but stable base line leading to low detection and quantification limits. Endcapped ODS gold column further reduces the requirement of buffers during chromatographic separation of especially basic drugs which ultimately enhances the working life of column, pump and flow cell.

MATERIALS AND METHODS

Chemicals and reagents

For this study HPLC grade Acetonitrile, hydrogen peroxide, hydrochloric acid and sodium hydroxide were purchased from Rankem Limited, India. Marketed preparation of Imeglimin Hydrochloride tablets were purchased from a local pharmacy shop. Gift sample of Imeglimin Hydrochloride was procured from Lupin Pharmaceutical. Water was acquired from a Milli-Q water (Millipore, USA). 0.22µ nylon membrane filter is used for filtration of sample and mobile phase.

Instruments and UHPLC conditions

A thermo fischer dionex UHPLC UltiMate 3000 series coupled with photodiode array detector (DAD-3000) and equipped with degasser were used for analysis. Chromeleon chromatography management system software was employed for data acquisition. Separation process was carried out using Hypersil gold ODS endcapped reverse phase column of dimensions (150x4.6 mm) with a particle of 3µ. This ultrapure, silica based endcapped column significantly reduces free hydroxyl group on silica thus reducing interaction of deprotonated hydroxyl group and amino moiety of drugs which is the major cause of peak tailing. Therefore, use of this superior column produces significant reduction in peak tailing, exceptional resolution, efficiency, accuracy, selectivity and sensitivity. Endcapped column further reduces the need of buffer during chromatographic separation of basic drugs which ultimately enhances the working life of column, pump and flow cell. Mobile phase consisting of water: acetonitrile in a ratio of (15:85% v/v) at a flow rate of 1 ml/min with an injection volume of 20 µl. was used. Detection wavelength was set at 240 nm and total run time of 10 min. For quantification the peak area response of Imeglimin was compared with corresponding calibration curve, wherein the peak area response of the calibration standards was plotted against their concentration.

Method optimization

To optimize chromatographic conditions different ratio of water, methanol and acetonitrile were tested in isocratic mode to achieve acceptable resolution, peak shape, efficiency and retention time. Finally, water: acetonitrile combination in ratio of (15:85) with the flow rate of 1 ml/min. with isocratic elution was selected because of greater optimized results. ODS and phenyl column were tried during the optimization of the method but the end capped ODS column relatively produces good symmetry and performance with a photo diode array detector. The wave length of 240 nm was selected to produce maximum absorbance and sensitivity. By using above condition, we get retention time of 3.831 minute for Imeglimin with tailing factor of 1.14. The number of theoretical plates for Imeglimin was found to be 3343 which indicates excellent column efficiency and the %RSD for the six replicate injections was found to be around 0.246. The developed approach suggested that it is highly precise and validated in accordance to ICH-Q2(R1) guideline.

Till today there is not a single stability indicating method for Imeglimin in the literature but only one reported method is developed for estimation of Imeglimin by HPLC [18]. The proposed UHPLC method can be utilized for routine analysis.

Preparation of standard stock solution

50 mg of accurately weighed quantity of Imeglimin is taken in 50 ml Volumetric flask and dissolved and diluted up to the mark with diluent and was ultrasonicated for 10 min for fully dissolving the content to get 1000µg/ml solution of Imeglimin and filtered through 0.22 µm membrane filter.

Preparation of mobile phase

The mobile phase of water and acetonitrile in the ratio of 15:85 (%v/v) was prepared by mixing 75 ml of water and 425 ml of ACN to get 500 ml of mobile phase.

Diluent

Water was used as diluent because of Imeglimin is soluble in it and not producing any asymmetry in chromatogram.

Method validation

The developed method was validated for various recommended parameters like specificity, linearity, accuracy, robustness, precision, Limit of Detection (LOD) and Limit of Quantification (LOQ) as per guidelines of ICH Q2(R1) [19, 21].

Degradation studies

Partial degradation of the drug was carried out using various forced degradation conditions on the Imeglimin standard. Proposed research has been carried out to develop a suitable analytical method which distinguishes pure drug peak from degrading products as per guidelines of ICH Q1A (R2). Additionally, the studies describe the conditions under which the drug is unstable, providing further information so that appropriate precautions can be taken during the process of formulation in order to avoid possible instabilities [20-22].

RESULTS AND DISCUSSION

The main analytical challenge during the development of a new method was to separate active Pharma ingredients. In order to provide good performance, the chromatographic conditions were optimized. Imeglimin belongs to a new category anti-diabetic drug called “glimins” and the stability indicating method for the drug using UHPLC has not been reported till date. So a new method has been developed and validated as per ICH Guideline. The results obtained for method development and validation suggest that the developed novel method for the selected drug is accurate, précised, specific and robust. The novelty of the method includes the utilization of endcap C_{18} column for the separation of drugs containing amino groups which enhances the complete resolution of the components. The usage of endcapped column reduces the instance of tailing effect which is a major limitation of separation of analytes possessing amino groups.

System suitability

In system suitability testing, USP tailing factor and plate count values were calculated by injecting standard solutions. These values were tabulated in table-1 and the standard chromatogram was shown in fig. 2.

Table 1: Results of system suitability

	Acceptance criteria	Imeglimin values
USP Plate Count	*NLT 2000	3343
USP Tailing	**NMT 2.0	1.14
USP Resolution	*NLL 2.0	-
% RSD	**NMT 2.0	0.246

n=6. *Not Less Than. **Not More Than

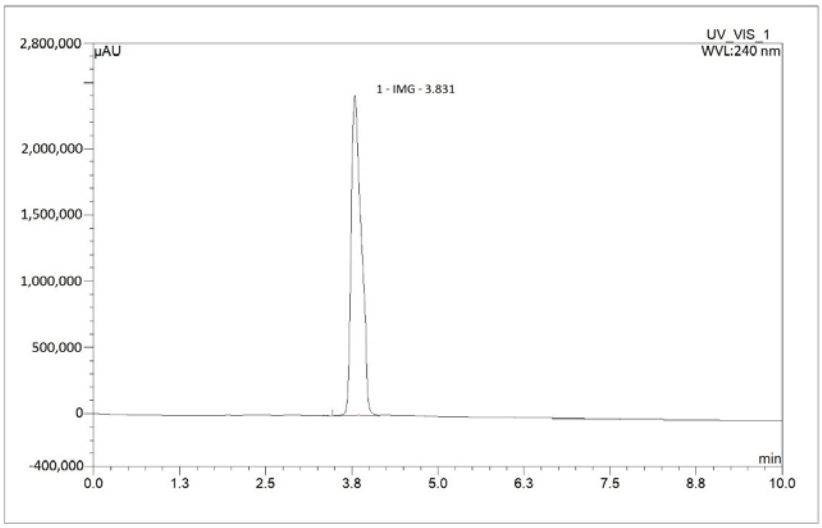

Fig. 2: Chromatogram of standard sample

Specificity

In this test method chromatogram of mobile phase blank, standard solutions and tablet samples were compared individually to examine the interference. The below fig. show that the active ingredients were well separated from blank and their excipients and there was no interference at the retention time of Imeglimin. Hence the method is specific.

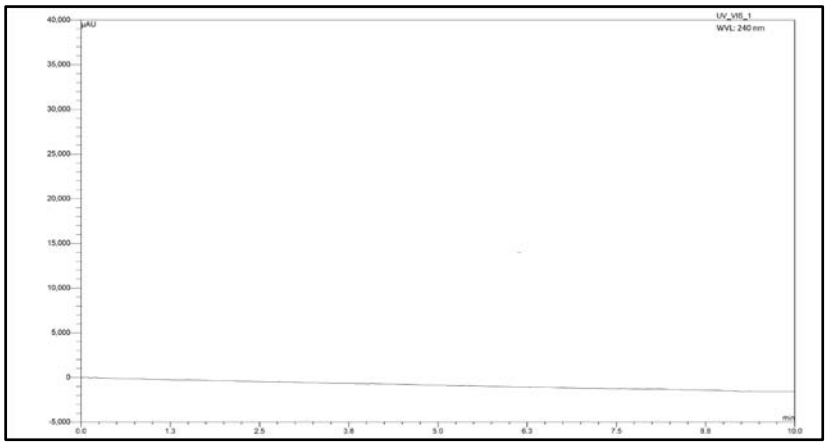

Fig. 3: Chromatogram for blank

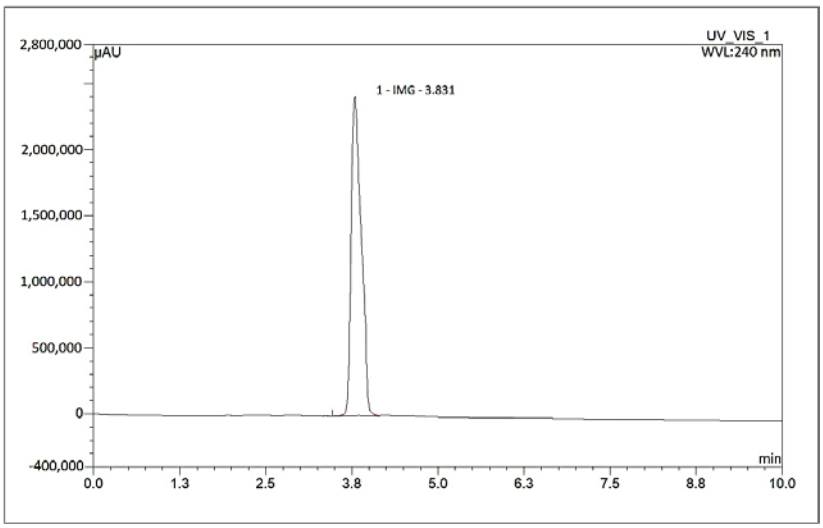

Fig. 4: Chromatogram for standard imeglimin

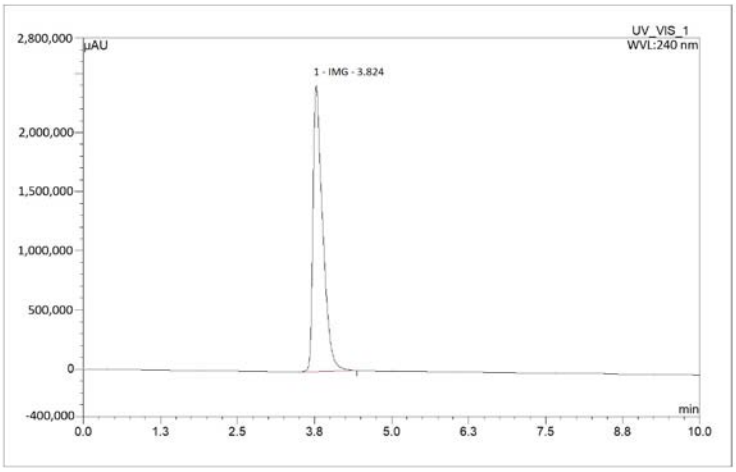

Fig. 5: Chromatogram for imeglimin tablet sample

Linearity

A series of solutions over the range of 25,50,100,150,200, 300 and 500µg/ml for Imeglimin were prepared and 20µl of each solution was injected into UHPLC system and peak area of the chromatograms were noted. Calibration curve was plotted against the concentration of the dilutions on the x-axis and corresponding peak areas on the y-axis. This will yield slope and intercept value of the regression line. Linearity was noted in the range of 25-500µg/ml. The regression equation is y=3199x+1605.5 with a correlation coefficient (R^2)>0.999, which shows excellent linear correlation. Linearity data are tabulated in table 2.

Table 2: Linearity of imeglimin

S. No.	Conc. (µg/ml)	Imeglimin area count
1	25	81593
2	50	161451
3	100	326672
4	150	471708
5	200	644784
6	300	963229
7	500	1600523

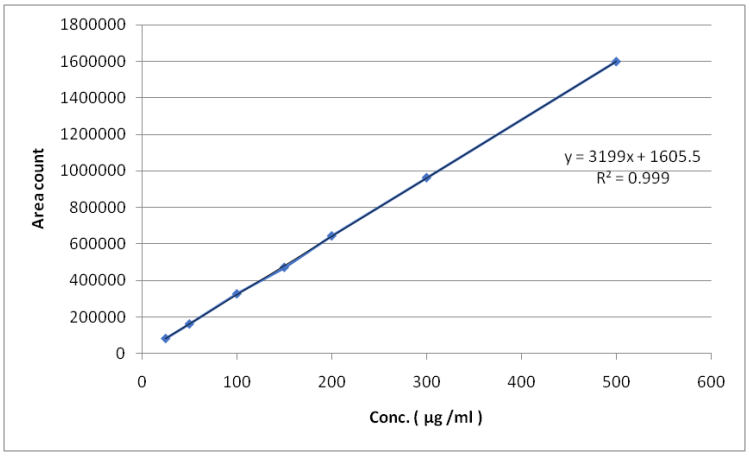

Fig. 6: Standard calibration plot of imeglimin at 240 nm

Precision

In method precision study prepare six replicated samples of same concentration of Imeglimin (200µg/ml) were injected into UHPLC system. Observed peak area values were used to calculate mean, SD and %RSD.

Intraday precision

Six replicates of a sample solution containing Imeglimin (200µg) were injected into UHPLC system and analyzed on the same day. Observed Peak area values were used to calculate mean, SD and %RSD values. It has been found that the proposed method produced very accurate results with %RSD value of 0.490, which was less than 2 percent of the maximum recommended value and percentage assay values near 100 percent. Results are given in table 3.

Inter-day precision

It is also called intermediate precision. In this method six replicates of a simple solution containing imeglimin (200µg) were analyzed on a different day. Peak areas were measured which were used to calculate mean, SD and %RSD values. The proposed method was found to be precise as the calculated %RSD value was (0.546), which was less than 2% and also the percentage assay values of imeglimin were close to 100%. The results are given in table 4.

Table 3: Intra-day precision results of Imeglimin

S. No.	Conc. (µg/ml)	Area count	% Assay
1	200	644789	100.5
2		641577	100.0
3		639669	99.7
4		642576	100.2
5		646609	100.8
6		647815	101.0
SD	0.492		
Mean	100.36		
% RSD	0.490		

n=6

Table 4: Inter-day results of Imeglimin

S. No.	Conc. (µg/ml)	Area count	% Assay
1		644228	100.4
2	200	641129	99.8
3		638280	99.5
4		646559	100.8
5		641213	99.9
6		647192	100.8
SD	0.541		
Mean	100.2		
%RSD	0.546		

n=6

Accuracy

Accuracy method was employed by the standard addition method in which standard addition of Imeglimin at three different concentration levels of 50%, 100% and 150% were performed in triplicate by adding 100µg, 200µg and 300µg of drug. Accuracy of the method is calculated by calculating the % recovery of the Imeglimin as per the ICH guidelines. Accuracy data are tabulated in table 5.

Table 5: Result of accuracy studies

S. No.	% Level	Imeglimin % recovery	% Assay
1	50	99.6	100.8
		100.7	
		101.1	
2	100	100.2	100.3
		100.5	
		100.2	
3	150	99.7	99.9
		99.1	
		101.0	

n=3

Robustness

Changing the method parameters like flow rate (±0.2 ml/min), mobile phase (±2%) and wavelength (±2 nm) determine the robustness of the selected method. A 200µg/ml solution of imeglimin was taken and 20µl was injected into the UHPLC system to measure its peak area for conducting a robustness study. Robustness data are tabulated in table 6.

Limit of detection (LOD) and limit of quantification (LOQ)

A limit of detection (LOD) and a limit of quantification (LOQ) were calculated according to the formula: -LOD = 3.3 σ/s and LOQ = 10 σ/s Where 'σ' is the standard deviation of the response and 's' is the slope of the calibration curve. LOD and LOQ determine the sensitivity of the developed method. The calculated value of LOD and LOQ were 0.04µg/ml and 0.14µg/ml, respectively.

Table 6: Result of robustness of Imeglimin

S. No.	Parameters	Conditions	*Mean peak area±SD (%RSD)
1	Flow Rate	(0.8 ml/min)	643691±1148(0.178)
2	Flow Rate	(1.2 ml/min)	642046±767(0.11)
3	Mobile Phase	(17:83)	647326±1963(0.30)
4	Mobile Phase	(13:87)	641990±2500(0.38)
5	Wavelength	238 nm	645930±1440(0.22)
6	Wavelength	242 nm	642116±2734(0.43)

n=3, *Mean peak area±SD (%RSD)

Table 7: Stability results of imeglimin

	Stability at RT		Stability at 2-8 °C	
	% Assay	% Deviation	% Assay	% Deviation
Initial	100	00	100	00
6 h	99.8	0.20	99.7	0.30
12 h	99.6	0.40	99.3	0.70
18 h	99.6	0.40	98.9	1.10
24 h	98.1	1.9	98.8	1.20

Stability

The stability testing of Imeglimin was performed by keeping standard solution samples at room temperature and between 2-8 degree Celsius. The experimental study showed that the drug solutions remained stable for at least two days at the mentioned storage condition. The results are tabulated in table 7.

Analysis of marketed formulation

The Content of Imeglimin in the LUPIMEG-500 mg tablets (Lupin) was determined by the proposed analytical method. Weigh and takes 20 Lupimeg tablets for trituration to get fine powder. Powder equivalent to 100 mg Imeglimin was taken in a 100 ml volumetric flask containing 50 ml of water. The flask is then ultra-sonicated for 20 min and make up the volume with water. The tablet sample solution was then filtered through a membrane filter and from the above solution, 2 ml is diluted to 10 ml with diluents so as to get 200µg/ml solution of Imeglimin for the assay. The final diluted sample is again filtered through 0.22µ membrane filter. 20 µl sample was injected into the UHPLC system to measure peak height, area and retention time. The assay value was calculated by assaying 6 samples of the Imeglimin tablets (Lupimeg). The average value was found to be 504 mg (100.8%) with a Standard deviation 0.584 and % relative standard deviation of 0.58. The obtained assay values were within the acceptable limit (98-102%) against the amount claimed in the LUPIMEG tablets. Results are given in table 8.

Table 8: Assay estimation of imegliminin lupimeg

Formulation	Label claimed (mg)	Amount found (mg)	Recovery %
Lupimeg-500	500	504	100.80

n=6

Stress degradation studies

The stability-indicating property of the developed RP-UHPLC method was carried out by stress studies as per ICH recommendation. Stress degradation of Imeglimin was carried out by forcefully subjecting the bulk sample into acidic, alkaline, oxidative, photolytic and thermal conditions. We studied the ability of the proposed developed method for measuring the analyte response in the presence of its degraded products due to stress applied in different conditions. All solutions utilized for this study were prepared with the stock solution. Results of Stress degradation studies are tabulated in table 9.

Acidic degradation

Acidic degradation was performed by taking 10 ml of stock solution in 50 ml volumetric flask and treating it with 10 ml of 0.1N HCl for 1 h in a thermostat maintained at 80 °C in laboratory conditions. The stressed sample was cooled, neutralized with 10 ml of 0.1N NaOH and then diluted with Mobile phase as per the requirement. 20µl was injected into the UHPLC system and its peak area was measured. 4.9% of Imeglimin degradation was observed in this study.

Alkali degradation

Alkali degradation performed by taking 10 ml of stock solution in 50 ml volumetric flask and treating it with 10 ml of 0.1N NaOH for 1 h in a thermostat maintained at 80 °C in laboratory condition. The stressed sample was cooled, neutralized with 10 ml of 0.1N HCl and then diluted with Mobile phase as per the requirement. 20µl was injected into the UHPLC system and its peak area measured. No degradation was observed in the study due to the high stability of the drug in alkaline medium.

Oxidation

20 ml of the stock solution was taken and transferred into 100 ml round bottom flask. The contents were then mixed with 80 ml oxidative agent (30% H_2O_2). The reaction mixture was kept to reflux at a high temperature (80 °C) for at least 2 h with intermittent shaking. Then the above solution was cooled and filtered. A volume of 20 µl was injected into the UHPLC system to measure peak height, peak area and retention time 10.7% of Imeglimin degradation was observed in this study.

Irradiation with ultraviolet light

100 mg sample powder of Imeglimim was exposed to UV light (254 nm) for 48 h in neat and clean surface. After the exposure period, the material was dissolved in 100 ml Mobile phase to get 1000 µg/ml concentration and filtered. It was suitably diluted to get 200µg/ml of Imeglimim and a volume of 20 µl was injected into the UHPLC system to measure peak height, area and retention time. 5.7% of Imeglimin degradation was observed in this study.

Thermal degradation

A sample powder of Imeglimim (100 mg) was exposed to thermal energy in a hot air oven at a temperature of 80 °C for 48 h. Then the sample material was dissolved in 100 ml Mobile phase to get the concentration of 1000µg/ml and filtered. Further dilution made to get of 200µg/ml Imeglimim standard solution. 20 µl sample solution injected into the UHPLC system to measure peak height, area and retention time. No degradation was observed in the study due to the high stability of the drug.

Table 9: Forced degradation results of Imeglimin

Degradation conditions	Imeglimin	
	% Assay	% Deg
Acid degradation	95.1	4.9
Alkali degradation	99.9	-
Peroxide degradation	89.3	10.7
Photo Degradation	94.3	5.7
Thermal Degradation	99.9	-

CONCLUSION

The proposed research work is found to be promising and less time-consuming with minimum amount of solvent utilization for method development compared to previous submitted research work. The developed method proved that the method is specific, accurate, precise, and robust for Imeglimin. Stress degradation studies revealed that Imeglimin withstands alkaline and thermal (high temperature) condition. At the same time, acidic, oxidative and photolytic degradation also occurred. The developed method and obtained statistical data manifested that designed protocol is simple, rapid and economical for the estimation of Imeglimin API and pharmaceutical formulation.

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Nil

AUTHORS CONTRIBUTIONS

All authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

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